Final Report

Mechanisms Underlying the Metallic-Like Conductivity of Microbial Nanowires

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SCIENTIFIC AND TECHNICAL OBJECTIVES

The overall objective of this research was to elucidate the mechanisms for the metallic-like conductivity of *Geobacter sulfurreducens* pili in order understand this novel biological phenomenon and to provide information for optimizing practical application of pili or pili mimetics. Specific short-term objectives were to: 1) investigate the mechanisms underlying metallic-like conductivity; 2) develop a structural understanding of the pili to probe the conduction mechanism at a molecular level; and 3) identify strategies for increasing the conductance of pili.

APPROACH

Electrical conductance of native pili attached to cells, as well as pili preparations, were studied with a diversity of biophysical and electrochemical methods, under conditions that were physiologically relevant whenever possible. A major shift in focus from previous studies was the study of charge propagation along individual pili still attached to cells with atomic force microscopy techniques. (b) (4)

The structure of pili was examined with high-resolution electron microscopy, X-ray diffraction, and synchrotron radiation, and modeled with three dimensional protein reconstructions.

RESULTS

In this project we: 1) demonstrated with advanced atomic force microscopy that, under ambient conditions, charges propagate in individual pili of *G. sulfurreducens* in a manner similar to that recently reported for carbon nanotubes; 2) documented a 3.2 Å spacing in wild-type *G. sulfurreducens* pili, expected for metal-like conductivity with synchrotron radiation, rocking curve X-ray diffraction, and electron diffraction; 3) reconstructed the three dimensional tertiary structure of pili with homology modeling, which further suggests the 3.2 Å spacing associated with metal-like conductivity; 4) demonstrated that individual pili can be chemically as well as electrostatically doped and show p-type transistor behavior with high mobility, revealing novel electronic properties of the pili with potential applications in bioelectronics; and 5) demonstrated that the *c*-type cytochromes on *G. sulfurreducens* pili are spaced too far apart for cytochrome-to-cytochrome electron hopping to promote electron transport along the pili. In addition to these major findings conductivity measurements were conducted that contributed to a number of other publications.

1. Geobacter sulfurreducens *pili function like carbon nanotubes*

We developed an electrostatic force microscopy method for evaluating charge propagation in individual pili of *G. sulfurreducens*, still attached to cells, under ambient, physiologically relevant, conditions. These key results, which were published in *Nature Nanotechnology*, demonstrated that: 1) under ambient, physiologically relevant, conditions pili still attached to cells function like carbon nanotubes - charge propagates in a manner consistent with metallic-like conductivity; 2) charge propagates in the absence of *c*-type cytochromes; 3) proton-doping and temperature responses are consistent with metal-like conductivity; 4) the lack of charge propagation in pili lacking key aromatic amino acids suggest that the metal-like conductivity can be attributed to the π-conjugation of aromatic residues; and 5) charges propagate through networks of physically connected pili. These properties are unprecedented for a biological material and have implications not only for current production in microbial fuel cells, but also other forms of extracellular respiration and cell-to-cell electrical communication. The potential for mass-producing a biological material, whose properties can be genetically manipulated, that has properties similar to carbon nanotubes is likely to have applications in the emerging fields of bioelectronics and nanobiomaterials.

2. Structural Insights into the Metallic-Like Conductivity of Pili

The structural features that contribute to the metallic-like conductivity of the *G. sulfurreducens* pili were evaluated with X-ray microdiffraction studies using synchrotron radiation (beamline) and rocking curve X-ray diffraction. These analyses revealed a characteristic 3.2 Å spacing in *G. sulfurreducens* conductive pili that is not observed in the poorly conductive pili of strain Aro-5. This spacing is consistent with overlapping pi-pi orbitals of aromatic amino acids. The 100-fold increase in conductivity that is associated with decreasing pH from 10.5 to 2 was accompanied by a comparable increase in the intensity of the 3.2 Å peak, further demonstrating a structure-function correlation between the 3.2 Å structure and the conductivity of pili.

However, conductivity is proportional to the number of charges in pili and thus it was considered that protonation of charged amino acids might also increase the net number of charges in pili. In order to further understand the protonation effect, the charge density of pili was computed as a function of protonation. Modeling showed that protonating the charged amino acids in pili can increase the charge density by only 10-fold. This is an order of magnitude lower than the observed 100-fold increase in conductivity, suggesting that an increase in the amount of charges due to protonation of charged residues cannot solely account for the higher conductivity. These modeling results are consistent with diffraction studies that demonstrated that protonation leads to conformational changes in the pili which is coincident with a dramatic increase in conductivity. These results were published in *mBio*, the premiere journal of the American Society for Microbiology.

3. Homology Modeling of the Structure of Geobacter sulfurreducens *Pili*

Homology modeling provided enhanced understanding of the structural features of *G. sulfurreducens* pili that contribute to conductivity. Several other laboratories had published homology models for *G. sulfurreducens* pili, which indicated that metallic-like conductivity was unlikely because aromatic amino acids were not packed closely enough together. However, those previous modeling efforts were based on the template of the *Neisseria gonorrhoeae* pilus, which is not appropriate because the *N. gonorrhoeae* pilin monomer shows poor homology to the *G. sulfurreducens* pilin monomer. In order to evaluate this further, a new template was developed based on the pilus of *Pseudomonas aeruginosa* strain K, which has a pilin monomer with higher homology to the *G. sulfurreducens* pilin monomer. The homology model based on the structure of the *P. aeruginosa* pilus predicted close packing of aromatic amino acids with distances of approximately 3-4 Å, consistent with the observations described above with X-ray diffraction methods. The results were published in *mBio*.

4. (b) (4)

5. *Demonstration that Cytochromes are Spaced Too Far on Pili for Electron Hopping*

There has been considerable debate whether *c*-type cytochromes associated with the pili of *G. sulfurreducens* promote electron transfer along the length of the pili via cytochrome-to-cytochrome electron hopping. This possibility was further evaluated with atomic force microscopy. The results clearly demonstrated that the cytochromes are spaced at distances much to far for cytochromes to account for electron conduction along the length of the filaments. These results were published in the high impact journal (IF=20) *Energy and Environmental Science*.

IMPACT/APPLICATIONS/TRANSISTIONS

The discovery of metallic-like conductivity in a biological protein filament is a paradigm shift in biological electron transport because it was previously considered that metallic-like conductivity would be impossible in biological materials. (b) (4)

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The understanding of the structural mechanisms for metallic-like conductivity, conferred by aromatic amino acids, (b) (4)

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